A Comparison of 4 Computer Based Root Diameter Measurement Systems – Efficacy with Diameters Less Than 0.5 mm

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Introduction

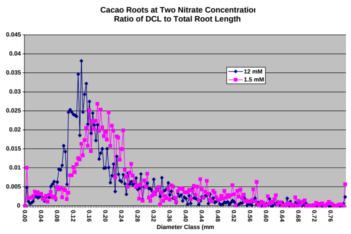
Current research on differential root development, as impacted by stress, suggests that there is a need for resolution of 10% shifts in diameter of 0.06 mm diameter and larger roots (Zobel, 2003, 2005a, b; Zobel et al., 2006). Existing software packages have not been checked for their efficacy at resolutions from 100 to 400 p mm^{-1}. However, curves representing the diameter class lengths of washed roots, imaged on a scanner at high resolutions (~250 p mm^{-1}) and analyzed with image analysis software, are very noisy (Figure 1). The basis for this noise has not been determined, nor have solutions or work-arounds been identified. This poster explores the source of some of this noise.

Abbreviations:

Pixels per millimeter: p mm^{-1}

Dots per inch: dpi (25.4 dpi = 1p mm^{-1})

Figure 1. Polygonal histogram of the results of a WR analysis of images (resolution = 252 pmm^{-1}) of roots from cacao plants grown at two nitrate concentrations (2 reps each). Note the extensive noise. DT and IP analyses produce very similar plots.



Materials and Methods

Plant materials were roots of *Theobroma cacao* L. (Cacao). The plants were grown in hydroponics with 5 levels of nitrate, roots harvested and one gram sub-samples measured for length and diameter.

Two different computer systems were used: a Dell Optiplex GX270 with a Pentium 4 at 3.2 Ghz running Windows XP Professional (DELL) and a 500 Mhz Power Mac G4 dual CPU, running OS X 10.4.2 (MAC). Four Software packages were used: WinRhizo , v 2005b (Regent Instruments) running on the DELL (WR); Delta-T Scan v 2.0 (delta-t.co.uk) running on the DELL in DOS mode (DT); Image Processing Tool Kit (IP) 5.0 (Reindeergraphics.com) running within Photoshop; and Photoshop CS (v. 8) running on the MAC. Results are commonly expressed as total length within each diameter (width) class (DCL). WR commonly reports this as centimeters, and DT and IP report it as mm. For some purposes, the Michigan State University, Root Image Processing Laboratory, analysis (RIPL - http://rootimage.msu.edu/root/index.php) was used.

The scanner used was an Epson Perfection 4870 PHOTO; non-interpolated image capability is 4800 x 9600 dpi (roughly 200 x 400 p mm^{-1}) (epson.com).

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Constructed Images

Images composed of lines of various thickness and angles were generated in Photoshop at 23.9 pixels per millimeter (23.9 p mm^{-1} - 600 dpi), 25 p mm^{-1} (635 dpi) or 400 p mm^{-1} (10160 dpi). Images were drawn in bit map format, and converted to 8 bit greyscale for rotation to different angles and for some analyses. Photoshop was used to bi-level threshold the greyscale images at specific threshold values prior to analysis or converting the rotated images back to bitmapped mode for storage. It is acknowledged that the software, WinRhizo, MSU-RIPL, and Delta-T scan, have been developed to account for roots crossing over and branching, and therefore results from analysis of simple lines may be less accurate.

Artifact Masking

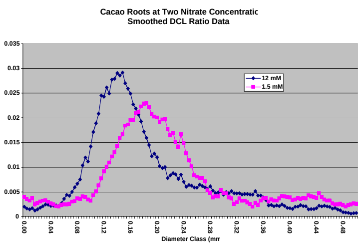
Because the noise in the analysis results (figures 1, 2) has an inherent pattern of dips and peaks that occur every 3 and 4 diameter class values, a five value running mean is a suitable smoothing algorithm to smooth the polygonal histograms normally used to report the results:

$$\hat{X}_i = (x_{i-2} + x_{i-1} + x_i + x_{i+1} + x_{i+2})/5$$

Results and Discussion

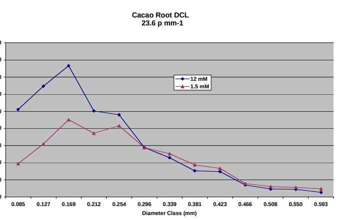
The two commercial root image analysis packages, WinRhizo and Delta-T Scan (WR and DT), and the commercial image processing package, Image Processing Tool Kit (IP) all produce results with noise (figure 1). Smoothing algorithms are effective for visually improving the histograms of Diameter Class Length (DCL) data (compare figure 1 with figure 2).

Figure 2. Smoothed data from figure 1. This demonstrates the separation of the peaks representing the diameter of the longest diameter class lengths for each treatment (1.5 mM = 0.187 mm and 12 mM = 0.151 mm, a difference of 0.036 mm or 20%).



The noise in these histograms is, however, not random. Peaks (and dips) occur every two, three or four diameter classes. Although they are amplified at higher resolutions, these artifacts are also visible at lower resolutions (figure 3).

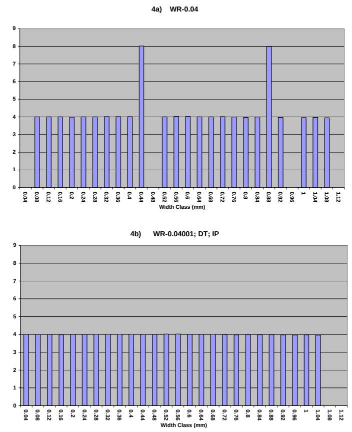
Figure 3. Cacao data from images made at a resolution of 23.6 p mm^{-1}. The differences between the two peaks is not obvious at this resolution. Note, both lines have a dip at 0.212 mm



At lower resolutions, however, the root diameter differences visible at higher resolutions are less obvious, or absent (compare figures 2 and 3).

WinRhizo has one artifact that does not occur with the other analysis packages (figure 4a). This artifact is easily obviated (figure 4b).

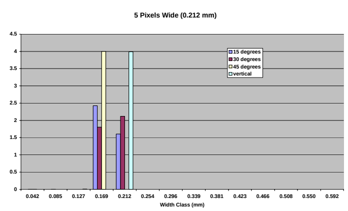
*Figure 4. Analyses of 26 vertical, 4.0 cm long, lines from 1 pixel to 26 pixels thick, drawn in Photoshop v. 8.0 with a pixel density of 25 p mm^{-1} (635 dpi). Figure 4a. is from a WinRhizo analysis with a Width Class of 0.040 mm, and demonstrates two artifacts introduced by the analysis: 1) the smallest line is represented by Width Class 0.08 rather than 0.04 (The saved data output classifies the first Width Class as 0.000<.L.<=0.040 when it should be 0.000<=.L.<0.040); 2) Width Class 0.48 (twelfth class from the left) is shifted back one pixel to Width Class 0.44, and Width Classes 0.92 and 0.96 are each shifted back one pixel each (0.96 = 0.48 * 2). Figure 4b is from a WinRhizo analysis with a base Width Class of 0.04001 mm, and demonstrates that this a simple fix, and eliminates the two artifacts seen in figure 4a. Figure 4b is also representative of unadjusted analyses with DT and IP.*



This shifting of pixels has a precise mathematical pattern: the 12th DC is reported with a thickness the same as the 11th, the 22nd and 23rd shifted to the 21st and 22nd, etc. For an extended plot it can be seen that the pattern is 1, 2, 4, 8, 16,,, times 12. This only occurs when the analysis is set at exactly the same resolution as the image was scanned in at or drawn. If the analysis is set at the scanned resolution plus 0.00001 mm, the problem disappears (figure 4b).

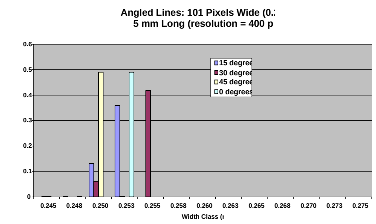
If constructed lines of precise thickness, length, and angle of rotation are analyzed with the software packages, reported line width can be off by a pixel, or a portion of the line reported as one width and the rest at a different width (figure 5).

Figure 5. Histogram of a WinRhizo analysis of four 40 mm long 5 pixel wide lines, each oriented at a different angle, drawn in Photoshop at 23.6 p mm^{-1}. Note that the analysis of the 45 degree line returned a value of 0.169 mm rather than the actual 0.212 mm width (shifted left). Also note that the 15 and 30 degree lines are both spread over two pixel classes. These lines correspond to the dip at 0.212 mm in figure 3.



At high resolutions, the splitting of a line into two widths can be amplified by reporting the widths as on either side of the true width (30 degree line figure 6).

Figure 6. A histogram of a WinRhizo analysis of four, 0.2525 mm wide; 5 mm long, lines oriented at different angles, drawn in Photoshop at a resolution of 400 p mm^{-1}. Note that one of the 30 degree bars is shifted up one pixel from actual and the other down one pixel; and the 45 degree bar is shifted down one pixel from actual leaving a gap at 0.2525 mm. Apparently, as resolution increases, the severity of the pixel shifts increases.



A pixel counting analysis of the lines for figure 5 confirms that the lines are of a uniform pixel width (table 1).

Table 1. Actual line width (z) for the lines analyzed in figure 5 are calculated by measuring x and y distances, in pixels, from one edge to the other side of the line, calculating z by: $z = (x * y)/(x^2 + y^2)^{0.5}$, then multiplying by 0.04233333 mm (the width/height of a square pixel when resolution is 23.6 p mm^{-1}).

	Line Width (mm)					
	Measurement #				Average	Nearest Pixel Width
Degrees Rotation	1	2	3	4		
0	0.212	0.212	0.212	0.212	0.212	0.212
15	0.204	0.203	0.202	0.205	0.203	0.212
30	0.223	0.218	0.211	0.218	0.217	0.212
45	0.209	0.209	0.209	0.209	0.209	0.212

Table 2 presents data that show that WinRhizo and DT-Scan both shift pixel widths of 45 degree angled lines. This type of analysis is not available for RIPL, and IP analyses correctly with one process and mimics WR and DT with the other (different processes not described here).

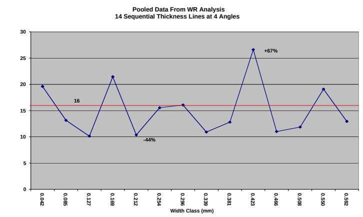
Table 2. Analysis of pixel shifts and length accuracy for an analysis of 14 accurate lines at four different angles by four different software packages. Resolution = 23.6 p mm^{-1}; width class 4 is 0.212 mm. WR and IP have the best length estimates.

Anal.	Deg.	Width Class (pixels) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Length
WR	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/- 2%
	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/- 2%
	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/- 2%
	45	-	Lft	n/a	-	Lft	-	n/a	Lft	Rgt	-	Lft	Rgt	-	-	+/- 2%
DT	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-25%
	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-5%
	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+10%
	45	-	-	n/a	Rgt	-	Rgt	n/a	-	Rgt	-	-	-	Rgt	-	+12%
RIPL	0															+/- < 1%
	15															~ +8%
	30															~ +8%
	45			n/a				n/a								+/- < 1%
IP	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/- < 1%
	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/- < 1%
	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/- < 1%
	45	-	-	n/a	-	-	-	n/a	-	-	-	-	-	-	-	+/- < 1%

Lft = a shift to one pixel narrower.
Rgt = a shift to one pixel wider.
n/a = unable to generate an accurate 45 degree line of this pixel width.

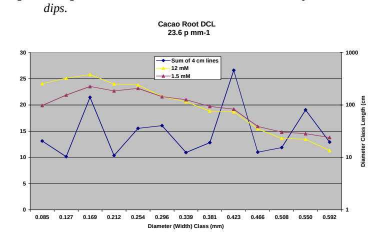
If 56 lines from 1 to 14 pixels thick and at 4 angles are analyzed and the results pooled by DC, the histogram shows that the pixel shifts and miss-measurements can have cumulative effects (Figure 7).

Figure 7. Polygonal histogram of the WR analysis represented in Table 2. All values for each width class were summed to get a total for each width class. Perfect analysis would give a value of 16 (red line) the highest peak was 67% higher than perfect, and the lowest dip was 44% less than perfect.



If the Cacao data from figure 3 is shifted to log scale and superimposed on figure 7 (see figure 8) the noise pattern is co-incident over all three independent polygonal histogram plots. If this was noise, it would be random and different for each plot; and would not be produced from constructed lines of exact thickness and angle.

Figure 8. Figures 7 and 3 combined. Note the co-incident peaks and dips.



In each of the above analyses, total root (line) length was estimated very accurately (+/- < 1%) by DT and WR.

Conclusions

- WinRhizo and Delta-T Scan accurately assess total length and average diameter.
- The tested image analysis software packages produce artifacts in diameter class analyses.
- At moderate to high resolutions, the cumulative deviations caused by these artifacts overwhelm treatment and genotype based diameter class length patterns. These deviations are only partially overcome (visually) by data smoothing.
- If root imaging at >12.5 p mm^{-1}, for root diameter class analysis, is to become routine, additional software development is necessary to eliminate these artifacts.

Literature Cited

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